

ABSTRACTS FROM THE ORIGINAL PAPERS.

*On the Enzymes contained in the Sap
of Ficus retusa.*

By Syôsuke TANAKA.

(Received, Oct 21, 1925.)

As a result of my experiments the sap of *Ficus retusa* contains two kinds of enzymes:— the oxidising and the proteolytic enzymes. Among the oxidising enzymes there have been qualified; oxidase, peroxidase and katalase; and the proteolytic ones contained, besides the coagulating enzyme, an enzyme like trypsin which acts upon proteins and decomposes it into peptone or albumose and finally converts them to amino compounds, in acid as well as in alkaline solutions.

*Vitamin-B Requirement of Different
Kinds of Yeast.*

By Raizo YAMAMOTO.

(Received, Oct 15, 1925.)

I. The author carried out a series of experiments to see whether there are some chemically defined substances besides vitamin-B or Bios, which accelerate the growth of yeasts. The substances tested were as follows:—

chlorophyll-a, chlorophyll-b, haemoglobin, haemin, quercetin, myricitrin, flavon of green tea, colouring matter of the cocoon of anthrae yamamai, tannic acid, gallic acid, pyrogallie acid, phloroglucin, vanillin, pyrrol, pyridin, quino-

lin, furfural, camphor, camphoroxime, cinnamic alcohol, cinnamic aldehyde, anisic aldehyde, benzaldehyde, nitrobenzene, o-nitro-naphthalene, picric acid unknown picrate from oryzanin, adenin and its picrate, nicotinic acid, β -acid, ethyl- and methyl-ester of β -acid, sodium salt of β -acid, adenyli-thiomethyl pentose, sulphuramino acid ($C_5H_{11}SNO_3$), cysitin, cysteine, cystin plus glutamin, tryptophane, uric acid, caffen semicarbazid, resin, resin (decomposed product of oryzanin), persimmon juice.

The yeasts used for the experiments were :-

Beer yeast Kirin, Wine yeast Nagasawa, Sake yeast (4-5 varieties), and distillery yeast. These were previously cultured on koji-agar.

As the liquid culture media, the Hayduch's and Nügeli's solution were used. The materials used for the preparation of these media were purified with utmost care, especially the cane sugar was repeatedly crystallized until it gave no blue colouration with ferri-ferri cyanid reagent.

The samples to be tested were added to the culture medium in the extent of 0.01-0.02%. One small loop of the yeast was distributed in 10c.c. of sterilized water, from which 2-3 loops were taken and introduced in each 10c.c. of the above named culture solution. For the control, koji- and malt-juice as well as the medium containing 0.01% of oryzanin were treated exactly in the same way, as above mentioned.

The inoculated solutions were kept at 28°C and the multiplication of the cells and the state of fermentation were carefully observed. When the fermentation was finished, the volume of the yeast, settled at the bottom, was compared with the control one.

The results of the experiments were almost negative. Only β -acid (dioxychinolin-carboxylic acid, obtained by the hydrolysis of Suzuki's crude oryzanin) its salts and esters exhibited a slight stimulating action upon beer and wine yeasts, though its action was far weaker than oryzanin itself. Picric acid was found to be slightly efficacious for the sake yeast.

II. It is well known that vitamin-B accelerates the growth and fermentative power of yeasts, though different kinds of yeasts behave quite differently towards it. Thus certain yeast growing at the bottom of the culture solution require much vitamin-B, while others growing on the surface of the same medium are found to be less influenced by it. The author made careful studies on this subject with the following 32 kinds (species or varieties) of yeasts, i. e.

Saccharomyces Sake ; 17 varieties

Saccharomyces Cerevisiae ; 5 "

Beer yeast Kirin

" " Yebisu

" " Froberg

" " Saaz (Unter-gärlige)

Saccharomyces Ellipseudius 6 varieties

Wine yeast Nagasawa

" " Yamanashi

" " Johannisberg

" " Oppenheimer

" " Charente Champagne

" " Albo

Saccharomyces Shausing 5 varieties

No. I

" II

" II (yellow)

" III

" IV

Distillery yeast

Saccharomyces Exigus

" Pasteurianus

" Ellipseudius

Zygosaccharomyces Major

" Soja

Willia Anomala

Pichia Farinosa

" Rōsa



As the source of vitamin-B, commercial "oryzanin" liquid (20 % alcoholic solution) was used, of which 0.005, 0.01 and 0.02mg. were added per c.c. of the culture medium.

For the determination of the fermentative activity, a special fermentation flask, constructed by Dr. U. Suzuki was used (J. Chem. Soc. Japan Vol. XLV, Nos. 5 & 6. June, 1925) It is a narrow necked flask of 100c.c. capacity, connected with a vertical CaCl_2 -tube the CO_2 evolved escapes through the tube, while the moisture being retained in it. On the upper side of the flask, there is a narrow bent tube, one end of which reaches nearly to the bottom of the flask, while the other end opens outside of the flask and is stoppered with rubber. This tube serves for the inoculation of yeast. (See Fig. I.) Each 50c.c. of the culture solution were filled in the flask, and 1c.c. of a dilute yeast suspension to be tested was inoculated to it. The flask was now kept in a thermostatt at 28° . During the experiment the whole apparatus being weighed at definite intervals of time. The decrease in weight corresponds to the quantity of CO_2 evolved. Thus the

percentage of sugar consumed by fermentation may be calculated and plotted in a curve.

Comparing these curves, the author observed the following facts:

1. Different varieties of sake yeast grew very rapidly in the medium containing 0.01 % of oryzanin, and decomposed the sugar almost completely in 7 days, while in the control medium containing no oryzanin, the growth was hardly visible until 5-6 days, but after that time they grew at the same rate with the former one. Sake yeast seems therefore to require only very little vitamin-B (if any) for their growth.

2. Beer yeasts grew generally very slowly in the vitamin free medium, only 1-5 % of the total sugar being decomposed in 2-3 weeks. By the addition of oryzanin, the growth was accelerated nearly in proportion to the amount of it added. They belong therefore to the class, which requires much vitamin-B.

3. Wine yeasts stand in the middle of sake and beer yeasts in this respect.

4. Distillery yeasts also required vitamin-B in fairly large amount.

5. *Saccharomyces Shausing*, which grows on the surface of the culture medium and forms scum on it, was found to be less influenced by vitamin-B.

6. *Willia anomala* grew well in the vitamin free solution, but the growth was somewhat better in presence of vitamin.

7. *Zygosaccharomyces major* and *soja* hardly grew even in the medium containing 0.02 % of oryzanin, but grew in koji or malt juice and strongly fermented, this variety therefore seems to require much vitamin.

8. *Pichia rosa* and *farinosa* did not require much vitamin.

From these results it may be concluded that the cultivated bottom yeasts generally require more vitamin for growth than the wild, top growing kinds do.